Strategies for reducing control group size in experiments using live animals

Matt Kramer, ARS, USDA
matt.kramer@ars.usda.gov
Enrique Font, Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universidad de Valencia

Outline

- Controls
- Borrowing information from historical controls
- When can historical controls be used?
- Other issues with controls

- Objective: Use fewer animals when performing experiments using live animals
- 3 R's (http://www.understandinganimalresearch.org.uk/how/three-rs/):

  - **Replace** the use of animals with alternative techniques, or avoid the use of animals altogether.
  - **Reduce** the number of animals used to a minimum, to obtain information from fewer animals or more information from the same number of animals.
  - **Refine** the way experiments are carried out, to make sure animals suffer as little as possible. This includes better housing and improvements to procedures which minimise pain and suffering and/or improve animal welfare.

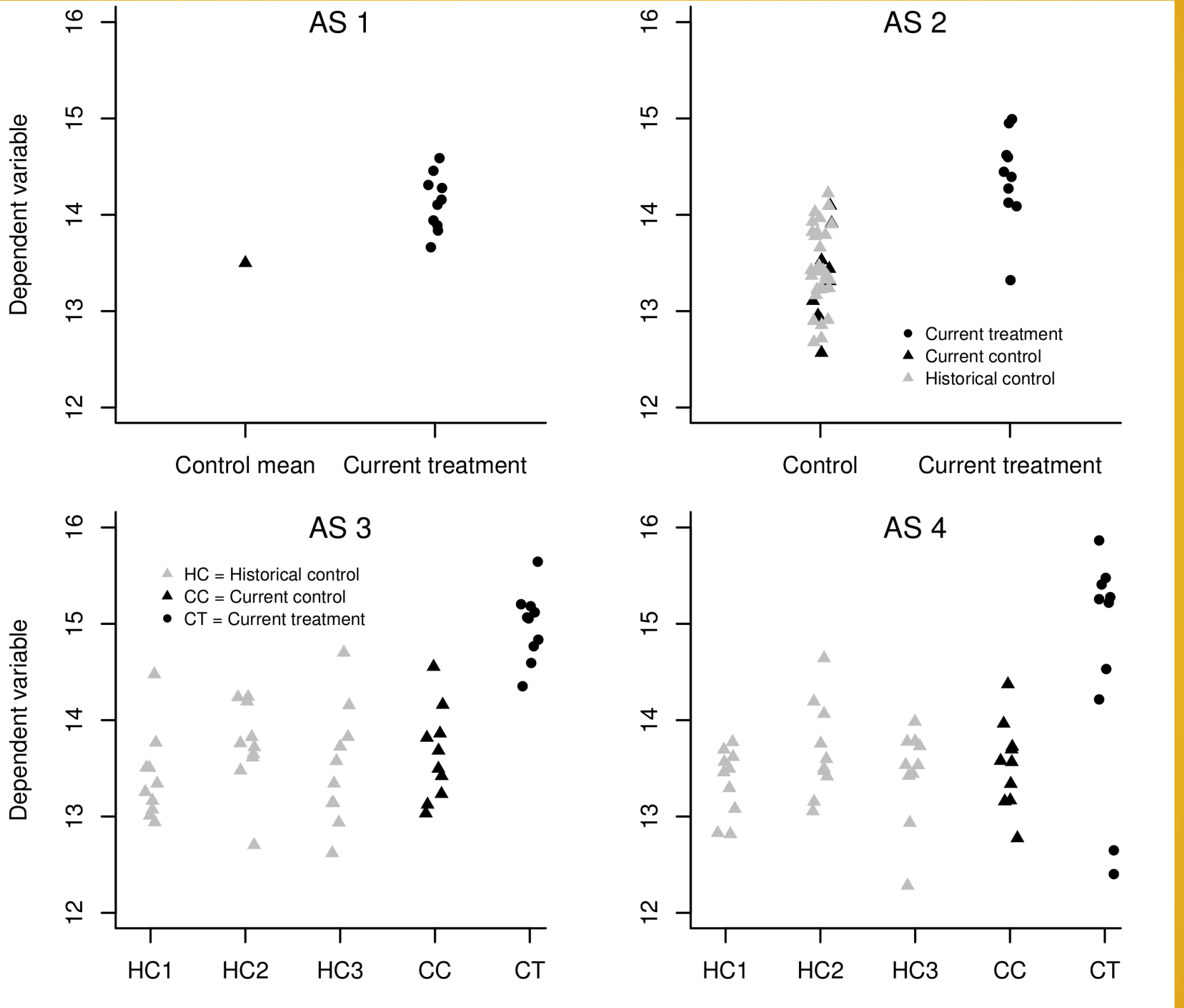
Whose responsibility is it to know how many and what kind of controls are needed?

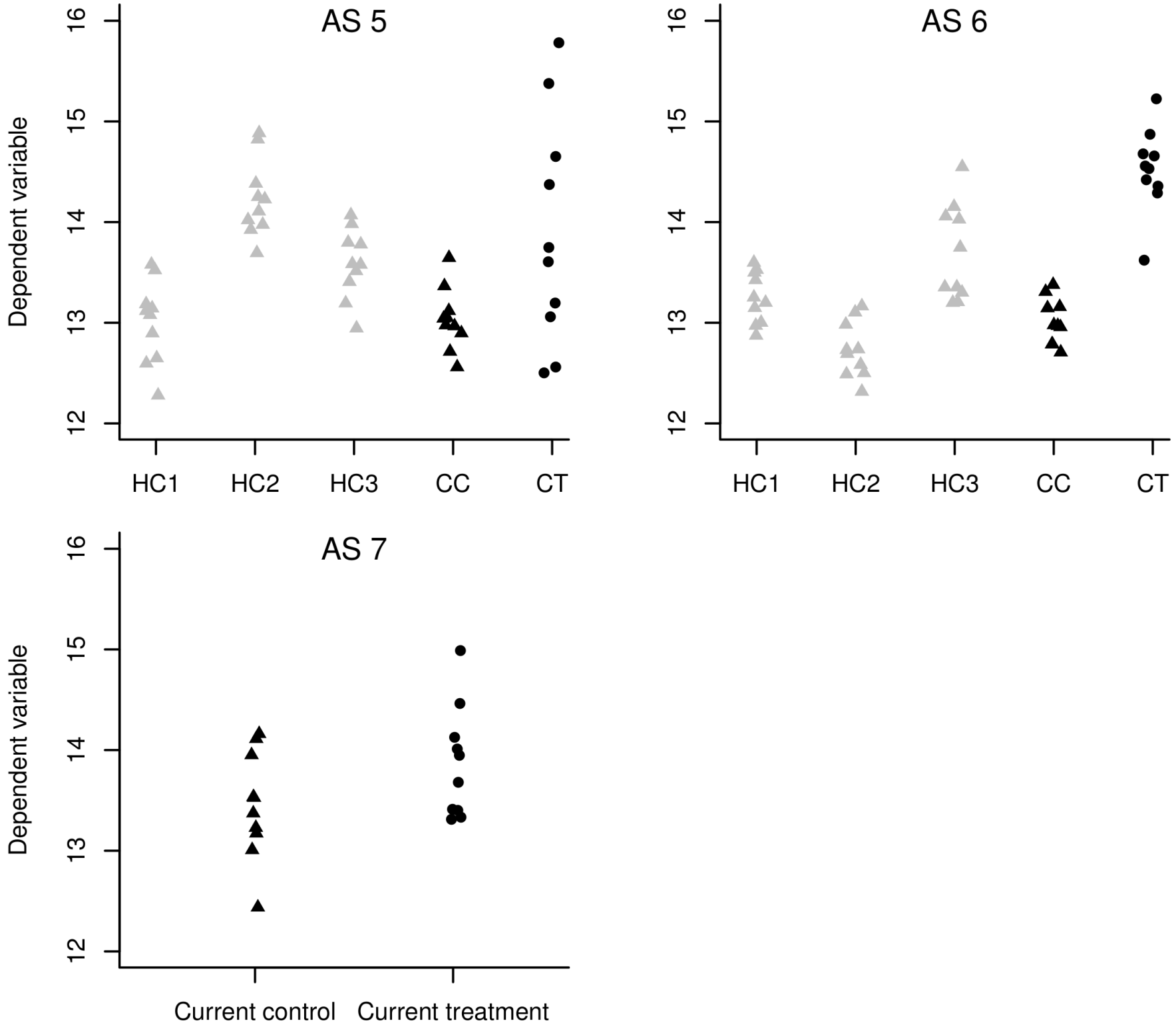
Problem: There is a void in the knowledge on the use of controls; neither scientists nor statisticians are exposed to the concepts during training. Prevailing view is to treat controls as just another treatment group.

Kinds of controls

- Negative control (animal remains in pre-experimental state)
- Positive control (pre-treatment applied to controls but not a subsequent treatment)
- Sham (== placebo) controls
- Concurrent vs. historical controls

We know far more about controls than we do about treated animals, but we ignore that information when we test a hypothesis using only current experimental subjects.


AS 1
Dependent variable
16
15
14
13
12
Control mean
Current treatment
AS 2
Control
Current treatment
Current treatment
Current control
Historical control
AS 3
HC = Historical control
CC = Current control
CT = Current treatment
HC1
HC2
HC3
CC
CT
AS 4
HC1
HC2
HC3
CC
CT


AS 5
AS 6
AS 7
Dependent variable
16
15
14
13
12
HC1
HC2
HC3
CC
CT
Current control
Current treatment

Incorporating previous control information

From strongest to weakest assumptions:

- Controls completely characterized (AS 1)
- Exchangeable (AS 2)
- Random experiment-to-experiment variability (AS 3 & AS 4)
- Borrow information for variances only (AS 5 & AS 6)
- Ignore historical controls (AS 7)

Model for mean	AS	Model for variance structure	test
$y_i = \mu_t$	1	$\widehat{\Sigma} = \sigma^2 \mathrm{I}$	$\frac{\mu_t - \mu_c}{\sigma/\sqrt{n}}$, μ_c fixed
$y_i = \mu_j; j = t, c$	2	$\widehat{\Sigma} = \sigma^2 \mathrm{I}$	$\frac{\mu_t - \mu_c}{\sigma_{tc}\sqrt{\frac{1}{n_t}+\frac{1}{n_c}}}$, where $\sigma_{tc} = \sqrt{\frac{(n_1-1)\sigma_t^2+(n_2-1)\sigma_c^2}{n_1+n_2-2}}$
$y_i = \mu_j + \gamma_l;$ $j = t, c_1, c_2, \ldots, c_k;$ $l = 1, 2, \ldots, k$	3	Each block (treatment or control group, here written for $n = 3$) has the form $\begin{bmatrix} \sigma^2 & \rho & \rho \\ \rho & \sigma^2 & \rho \\ \rho & \rho & \sigma^2 \end{bmatrix}$, where current observations from $j = t$, c_1 are in block $l = 1$; each historical control group forms its own block, ρ is an estimated within-block covariance, it is 0 for observations from different blocks.	$\frac{\mu_t - (\mu_{c1} + \ldots + \mu_{ck})/k}{\sqrt{\boldsymbol{a}'\left(\boldsymbol{X}'\widehat{\Sigma}^{-1}\boldsymbol{X}\right)^{-1}\boldsymbol{a}}}$, where $\boldsymbol{a}$ is the contrast vector, here $\left(1, \frac{-1}{k}, \frac{-1}{k}, \ldots\right)'$, $\boldsymbol{X}$ is the design matrix, and $\widehat{\Sigma}$ is the estimated variance-covariance matrix for the y_i observations.
$y_i = \mu_j + \gamma_l;$ $j = t, c_1, c_2, \ldots, c_k;$ $l = 1, 2, \ldots, k$	4	Historical control groups as for AS 3, current treatment and control groups as $\begin{bmatrix} \sigma_t^2 & \rho & \rho & & & & \\ \rho & \sigma_t^2 & \rho & & 0 & & \\ \rho & \rho & \sigma_t^2 & & & & \\ & & & \ddots & & & \\ & & & & \sigma^2 & \rho & \rho \\ & 0 & & & \rho & \sigma^2 & \rho \\ & & & & \rho & \rho & \sigma^2 \end{bmatrix}$, where variances of observations of the treatment group differ from those of current and historical controls.	Same as AS 3 except $\widehat{\Sigma}$ differs (see variance structure).
$y_i = \mu_j + \gamma_l;$ $j = t, c_1, c_2, \ldots, c_k;$ $l = 1, 2, \ldots, k$	5	Same as AS 4	$\frac{\mu_t - \mu_{c1}}{\sqrt{\boldsymbol{a}'\left(\boldsymbol{X}'\widehat{\Sigma}^{-1}\boldsymbol{X}\right)^{-1}\boldsymbol{a}}}$, where $\boldsymbol{a}$ is the contrast vector, here $(1, -1, 0, \ldots, 0)'$
$y_i = \mu_j;$ $j = t, c_1, c_2, \ldots, c_k$	6	$\sigma^2 \mathrm{I}$	Same as AS 5 except $\widehat{\Sigma}$ differs (see variance structure).
$y_i = \mu_j; j = t, c_1$	7	$\sigma^2 \mathrm{I}$	Same as AS 2 but only the current control group is used.

Number of current controls	Number of current treated	Number of historical controls per group	Number of historical control groups	Power for a fixed control mean (AS 1)	Power assuming stable control means and equal variances (AS 3)	Power assuming stable control means and unequal variances (AS 4)	Power assuming unstable control means and unequal variances (AS 5)	Power assuming unstable control means and equal variances (AS 6)	Power ignoring historical controls (AS7)
3	5	5	4	0.62	0.65	0.65	0.42	0.42	0.34
3	5	5	3	0.61	0.64	0.62	0.41	0.41	0.34
3	5	5	2	0.61	0.60	0.61	0.40	0.40	0.34
3	5	5	1	0.62	0.55	0.54	0.39	0.39	0.34
6	5	5	3	0.61	0.67	0.67	0.56	0.55	0.50
9	5	5	3	0.61	0.71	0.71	0.63	0.63	0.59
12	5	5	3	0.61	0.73	0.72	0.67	0.67	0.65
15	5	5	3	0.61	0.75	0.75	0.71	0.71	0.69
3	10	5	3	0.96	0.80	0.79	0.49	0.49	0.44
3	15	5	3	1.00	0.86	0.86	0.53	0.53	0.50
4	5	5	3	0.60	0.65	0.64	0.47	0.47	0.40
3	5	3	3	0.61	0.59	0.59	0.40	0.40	0.35

strongest ⟷ weakest

assumptions

Results

Power increases if historical controls are used, the savings in animals depend on what information is borrowed (model assumptions).

For example, if borrowing both mean and variance information, 3 current + 20 historical controls yields the same power as 12 current controls.

When to use historical controls

- Series of experiments using the same control conditions
- Same lab, same species, possibly same source for animals
- No evidence of drift
- Control chart methodology to track controls
- In our simulations, a 2:1 (historical:current) is conservative, but not generalizable. There is no software currently available that determines power for the various sets of assumptions (i.e. for sample size determination).

Other control group issues

- Designs
- Heterogeneous variances
- No controls recover
- Controls for rare events

Recommendations

- Verify that the experiment requires controls
- If so, consider strategies that reduce the number of controls (e.g. subjects serve as their own controls)
- Are variances lower in controls?
- Consider using historical controls. If so, how much information should be borrowed?
- For additional details, see: Kramer, M. and E. Font. *In press*. Reducing sample size in experiments with animals: historical controls and related strategies. Biological Reviews doi: 10.1111/brv. 12237, downloadable from: https://www.ars.usda.gov/northeast-area/people/matthew-kramer/